RESISTANCE IN TOMATOES TO A VIRUS COMPLEX

by

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INTRODUCTION

In the summer of 1952 the olericulturists of Kansas State College observed many tomato plants with a peculiar malady. These plants were found in both home vegetable gardens and commercial plantings so it was not confined to any particular variety or source of plants. The symptoms were similar to those found on plants infested with cucumber mosaic virus.

This problem was concerned with screening numerous species and horticultural varieties of tomatoes for resistance to this disease. Since the symptoms of this disease have been reported previously by numerous workers, they are not reported again in this paper. The degree of resistance was the prime concern here since the resistant species or varieties were to be incorporated into a tomato breeding program designed to obtain resistant strains to this and other diseases.

Since no practical method of control is known for this disease, it was hoped by screening the commerical varieties that some adapted variety might be found that was resistant to this disease. The different species of the genus Lycopersicon were screened so if any resistance was found these species could be used in the breeding program.

LITERATURE REVIEW

Much of the literature reviewed here was not specifically on tomatoes due to the scarcity of literature on this virus complex in tomatoes. The reviewed material was on other horticultural crops and was thought applicable here.

Economic Importance

Holmes (1939) reported that 133 plants belonging to 34 plant families had been found susceptible to cucumber mosaic virus and 125 plants belonging to 27 plant families were susceptible to tobacco mosaic virus. Included in this list were many of the common garden vegetables, annual and perennial flowers, agronomic crops and weeds. These plants were not confined to any specific area of the world but were grown throughout the cultivated areas of the world. He also listed many synonyms for both of these viruses many of which have been found frequently in the literature.

Smith (1946) wrote that the three most frequently encounted viruses in vegetables are: tobacco mosaic virus, cucumber mosaic virus and the spotted wilt virus. He also stated that the tomato is probably the most susceptible vegetable to these viruses.

Dimock (1943) in discussing virus diseases of greenhouse crops listed tobacco mosaic, cucumber mosaic and spotted wilt viruses as the most frequently encountered viruses and that most greenhouse crops were susceptible to the first two of these viruses. He warned greenhouse operators who grew both

vegetable and bedding plants because of the ease of transmitting these diseases mechanically. He also cautioned that smoking and chewing tobacco could spread tobacco mosaic and should be prohibited around these plants.

Symptoms

Johnson (1927) wrote:

> ...Nowhere in the realm of plant pathology are symptoms of less value in description than in the plant virus diseases, because of the remarkable influence of enviromental factors, and the possible co-existance of two or more viruses in a single plant.

He stated later that the proper comparative host studies were sufficient in most cases to determine the viruses and in some cases proved the only available method and continued by listing symptoms of tobacco mosaic and cucumber mosaic virus diseases on several of there hosts.

Smith (1946) wrote:

> ...It must be remembered that viruses are disease agents which are below the limit of vision and almost the only criterion of their existance is the reaction of the host to infection. The symptoms of a plant virus disease therefore are of major importance in the indentification of the virus concerned.

Mogendorff (1930) worked with a virus complex. A combination of tobacco mosaic and cucumber mosaic in tomatoes, and reported that diagnosis on the basis of symptoms was very difficult, if not impossible, if both viruses manifest their symptoms simultaneously on the same host plant. In that case their symptoms will be superimposed and the presence of tobacco mosaic is difficult to detect nless inoculation experiments are performed.

Cucumber Mosaic Symptoms The symptoms reported in this paper have been compiled from those reported by numerous workers and were not placed in the order of their appearance on the plant. Those reported were: mottling, narrowing of the leaf blade, stunting, twisting of the growing point in a corkscrew fashion, rolling, twisting and folding of the leaflets, excessive number of leaflets, chlorsis, and malformation of the fruits. Occassionally the extreme reduction or absence of the lamina to give the fern-leaf or shoestring type of symptoms.

Tobacco Mosaic Symptoms Like the list of symptoms for cucumber mosaic these symptoms were compiled from those listed by numerous workers. They were: mottling, stunting, blistering or raised areas in the leaf blade, malformation of leaflets, rolling or folding of leaflets along the midribs, chlorsis, and the development to the lobes of the leaflets into spine like structures.

Incubation Period

Johnson (1927) wrote:

> ...The incubation period for any one virus naturally varies greatly depending upon the condition of the host, other environmental factors, relative susceptibility of the host and source of inoculum.

Mogendroff (1930) reported the incubation periods for tobacco mosaic and cucumber mosaic viruses on tomato seedlings: Tobacco mosaic- 10 days at 18-23° C and 6-7 days at 25° C; cucumber mosaic- 10-17 days at 18-23° C and at higher soil tempertures, 30-35° C, the symptoms were masked and did not

appear.

Smith (1946) reported that symptoms of tobacco mosaic 10-14 days after inoculation on tomatoes and symptoms of cucumber mosaic were most pronounced between the setting of the first and second clusters of fruit.

Kikuta and Frazier (1947) wrote that the first symptoms of tobacco mosaic on tomato seedlings appeared in 5 days after inoculation.

Factors Affecting Symptoms

Mogendorff (1930) in reporting his findings stated that symptom production was dependent upon: age of plant at the time of infection, atmospheric temperature and method of inoculation.

Dimock (1943) reported that the earlier the infection took place the more severe the symptoms were on the plants.

Light Intensity Mogendorff (1930) found that 2000 watts of artifical light for eight hours at 28-30° C. and 60 percent relative humidity after inoculation gave the most pronounced symptoms in his tests.

Pound and Cheo (1952) in reporting their findings with cucumber mosaic in spinach found that light intensity of 400 foot-candles for sixteen hours produced more severe symptoms than 1600 foot-candles for sixteen hours.

Bawden and Roberts (1948) reported that shading bean and Nicotiana glutinosa plants for 24 to 48 hours prior to inoculation increased their susceptibility to tobacco mosaic.

Elmer[1] in conversation with the writer stated that tomato plants shaded for 24 hours prior to inoculation were more susceptible to both cucumber mosaic and tobacco mosaic.

Temperature Cheo and Pound (1952) reported that both air and soil temperatures effected the concentration of cucumber virus 1. in spinach and tobacco. In the case of tobacco they found that low temperatures followed by high temperature and again by low temperature coincided with a build up of the virus concentration and symptom production, then a sudden drop of virus concentration and a partical recovery period followed by a secondary peak of virus concentration and symptom production.

Pound (1952) in reporting his finding on cabbage resistance to a virus complex stated that temperature had a direct effect on it. He found some cabbage was resistant at 24° C. and below, however; the resistance broke down at 28° C. and above.

Pound and Cheo (1952) reported in their studies on resistance to cucumber mosaic in spinach that temperature had a marked effect on susceptibility and resistance. When both soil and air temperature were maintained at 16° C. it required five times as long to kill a susceptible plant as it did when both temperatures were at 28° C. All resistance broke down at 28° C. and above in so called resistant plants.

Mogendorff (1930) found that 18-22° C. was the optimum

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1. Elmer, O. H. Plant Pathologist at Kansas State College.

temperature for symptom production on tomatoes by both cucumber mosaic and tobacco mosaic.

Nutrients Cheo et al (1952) reported in their studies on the relation of host nutrition to concentration of cucumber virus 1. in spinach that the virus concentration was directly related with the growth of the host. They found that the nutrient levels that gave the opimum growth yielded the highest virus concentration.

Roberts (1952) reported that both tobacco mosaic and cucumber mosaic viruses moved independent of food and mineral movement or translocation.

Selman (1946) found that plants inoculated with tobacco mosaic after they had started to set fruit contained the virus principally in the fruits. The plants rarely showed symptoms on the growing point unless some condition checked the flow of nutrients and carbohydrates into the fruit and started the reverse process where the plant was required to draw on this reserve.

Sources of Inoculum Sill and Walker (1952) in reporting their findings stated that extracts from cucumber, watermelon and spinach plants were highly inhibitive to cucumber mosaic virus and those from muskmelon, squash, pumpkin, cowpea, tobacco, tomato and Nicotiana glutinosa were moderate or slightly inhibitive.

Johnson (1927) reported tobacco extract was a good source of inoculum for cucumber mosaic but that pokeweed and potatoes were poor sources.

Slagg[1] in conversation with the author stated that extract from healthy potato plants added to the extract from tomato plants infected with cucumber mosaic gave more severe symptoms on tomatoes than the tomato extract alone.

Elmer[2] in conversation with the author stated that local lesions on the leaves of Nicotiana glutinosa were a good source of tobacco mosaic and that Nicotiana glutinosa when infected systemically with cucumber mosaic provided a good source of inoculum.

Methods of Inoculation

Bawden (1950) listed the main methods of virus inoculations as: intergrafting and dodder, sap inoculation and insects. He qualified this by adding that all three methods had not been successful with all viruses. Cucumber mosaic has been successfully transmitted by all three methods. On some of its hosts tobacco mosaic had been transmitted by all three methods.

Roberts (1950) reported that tomato plants had been infected with tobacco mosaic, potato X virus and tomato bushy stunt by root inoculations. He listed his methods of inoculations as: (1) fibrous roots were rubbed with the fingers moistened with infective sap and celite and then washed the

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1. Slagg, C. M. Plant Pathologist at Kansas State College.
2. Elmer, O. H. Plant Pathologist at Kansas State College.

roots with water, (2) same as 1, except the top was cut off immediately above the two seedling leaves, (3) the tap root was tied off with a thread half way down and the lower portion inoculated with a flattened needle, then the area washed, (4) same as 3, except the roots were cut off and put in distilled water in the dark. Mention was also made of adding the inoculum to the soil and cultural solution which resulted in root infection but rarely top infection. He found that the surface roots and tap roots gave a higher percentage of infection than the fibrous roots.

Fulton (1941) found that roots of tobacco plants were easily infected by direct inoculation but that the tobacco mosaic virus moved upward very slowly and that it rarely or only belately entered the stem or induced leaf symptoms. When the virus entered the stem it took a long time to cause leaf symptoms. On tomatoes inoculated with tobacco mosaic through the roots he found a high concentration in the roots but it rarely moved up into the tops and produced symptoms there.

Bawden (1950) stated that tobacco could be inoculated by adding the inoculum to the soil.

Viruses in the Seed

Smith (1946) reported that seed transmission was comparetively rare and that the reasons were not known.

Raychaudhuri (1952) found that tomato seeds retained tobacco mosaic for a period of twenty seven days. After this period, plants obtained by the germination of previously

infected seeds showed no symptoms of tobacco mosaic.

Dimock (1943) reported that tobacco mosaic is seed borne in petunias and some weeds.

Resistance Reported

Kikuta and Frazier (1947) reported resistance to tobacco mosaic in Lycopersicon hirsutum. They found that this resistance was not completely dominant since no F_1 L. esculentum x L. hirsutum hybrid had shown any degree of tolerance. They also reported that several segregates from a cross of L. esculentum (HES - 2269) x (L. peruvianum x Michigan State Forcing x L. pimpinellifolium) x L. hirsutum showed high degrees of tolerance to tobacco mosaic.

Alexander and Hoover (1953) reported they found resistance to tobacco mosaic in twenty seven strains of L. peruvianum and some resistance in four strains of L. hirsutum.

Frazier and Dennett (1949) stated that they had found a high dominance of resistance in some complex hybrids which indicated possible ultimate value for use in commercial F_1 hybrid combinations.

MATERIALS AND METHODS

The tests reported here were conducted in the horticulture research greenhouses at Kansas State College. Of the genus Lycopersicon, eighty two species and horticultural varieties were tested. The plants were grown and inoculated by two different methods.

Production of Plants

Seedlings All plants used in this problem were grown from seed in the horticulture greenhouses. The seeds were obtained from various sources (Table 1.) and were sown in five inch clay pots containing sterilized sand 15 to 30 days[1] prior to the inoculation date.

Flats The flats were of the standard greenhouse type and measured four inches by sixteen inches by twenty two inches. They were filled to within one half inch of the top with soil and sterilized prior to planting. The flats were divided lengthwise into two equal sections and a different species or variety of tomato was grown in each section. The plants in these sections were spaced two inches by three inches. The rows ran parallel to the short axis of the flat with the terminal plants in each row being one and one half inches from the side of the flat. This spacing allowed twenty five plants per section. The inner row of each section

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1. Wild species were slower to germinate and to grow so required a longer period of growth before they were large enough to transplant.

Table 1. Source, name and accession number assigned.

Source: Primary Plant Introduction Station Ames, Iowa

Variety or Species name	Sender's stock or pedigree number	Accession No. assigned
L.* esculentum	PI 109315	1
	PI 109835	2
	PI 119105	3
	PI 124135	4
	PI 127823	5
	PI 128446	6
	PI 129049	7
	PI 177008	8
	PI 180234	9
L. esculentum var. chili	PI 128609	10
L. esculentum X L. pimpinellifolium	PI 118409	11
	PI 119214	12
	PI 128194	13
	PI 129027	14
L. glandulosum	PI 126434	15
	PI 126440	16
L. hirsutum	PI 126445	17
	PI 127827	18
L. hirsutum var. glabratum	PI 126449	19
	PI 134417	20
L. peruvianum	PI 126431	21
	PI 126935	22
	PI 128648	23
	PI 129135	24
	PI 129152	25
L. peruvianum var. glabratum	PI 127829	26
L. pimpinellifolium	PI 79532	27
	PI 112215	28
	PI 126929	29
	PI 126953	30

* Lycopersicon

Table 1. (con't)

Source: Primary Plant Introduction Station Ames, Iowa

Variety or Species name	Sender's stock or pedigree number	Accession No. assigned
L. pimpinellifolium	PI 128639	31
	PI 129156	32
	PI 143522	33
	PI 143679	34
	PI 144955	35
	PI 190256	36
Marker-gene accessions	PI 193399	37
	PI 193400	38
	PI 193401	39
	PI 193404	40
Source: Corneli Seed Company, St. Louis 2, Missouri		
Bonny Best	3841	41
Certified Grothen's Globe Strain No. 2	2533	42
Louisiana Dixie	2520-2	43
Gulf State Market	2530-1	44
Oxheart	2532-1	45
Sunray	6342	46
Certified Indiana Baltimore	2534	47
Dwarf Champion Tree Tomato	5332	48
Yellow Pear	10181	49
Manosota	2522-1	50
Wisconsin	1828	51
Stokesdale		52
Victor or Bounty		53
Ponderosa		54

Table 1. (con't)

Source: Corneli Seed Company, St. Louis 2, Missouri

Variety or Species name	Sender's stock or pedigree number	Accession No. assigned
Keystone 40-46	6511	55
Earliana	2521-2	56
Valiant	5020	57
Rutgers	4962	58
Break O'Day	2531-2	59
Certified Pan American	8827-3	60
Prichard	5062	61
Marglobe	4752	62
Garden State Improved	2527-1	63
Sioux Early Red	8891	64
Urban	2537	65
Source: W.A. Atlee Burpee Company, Clinton, Iowa		
Rutgers	1072c	66
Source: Associated Seed Growers, Indianapolis, Indiana		
Wisconsin-55		67
Source: Peto-Hollar Company, Rocky Ford, Colorado		
49-51		68
Source: University of New Hamsphire, Durham, New Hamsphire		
Double Rich		69
Source: Purdue University, Lafayette, Indiana		
Tippecanoe		70

Table 1. (concl.)

Source: Cornell University, Ithaca, New York

Variety or Species name	Sender's stock or pedigree number	Accession No. assigned
Val North		71
Source: South Dakota State College, Brooking, South Dakota		
South Dakota 65		72
Source: Southern Great Plains Field Station, Woodward, Okla.		
Western Red		73
Source: Grand Rapids Growers, Grand Rapids, Michigan		
Certified Marglobe	160-c	74
Prichard		75
Source: Francis C. Stokes & Company, Vincentown, New Jersey		
Valiant		76
Stokes Cross #2		77
Stokes Cross #5		78
Master Marglobe	MN 62	79
Source: Joseph Harris Company Inc., Rochester 11, New York		
Queens	877	80
Fireball	861	81
Van Cross	891	82

was retained as a check row so that the two check rows were always side by side in the center of the flat. In the first trials all plants of the orginal planting that died within the first week were replanted but in later trials additional plants were placed in each row in order to reduce the difference in size and growth rate. This gave all plants identical conditions and prevented older plants from shading the smaller young plants.

Bench A portion of the west bench in the vegetable research greenhouse was used for this problem. This bench was a cement ground bench measuring ninety five feet long, six feet wide, and three feet deep of which thirty inches are above the ground level. The longitudinal axis of the bench ran north and south. The section of the bench used was the north thirty feet. The plants of those varieties or species that were grown in the bench were spaced six inches between rows and one inch within the row. The check plants were placed at the start of each row directly behind the name stake and all inoculated plants behind them. Seven check plants and fifty plus inoculated plants if available were planted into each row.

Inoculum

Identification By the use of established indicator plants the identifying symptoms of the specific viruses were found. The causative agency was a virus complex which consisted of a strain of cucumber mosaic virus and a strain of

of tobacco mosaic virus.

The Source of Inoculum The inoculum was obtained from cuttings made from a tomato plant which was growing in a garden located southwest of Manhattan, Kansas. These cuttings were rooted and grown in six inch clay pots in order that the same source of infection would be available.

Method of Preparation The inoculum for both methods of inoculation was prepared by grinding infected plant tissue with a mortar and pestle, then straining the macerated tissue through cheese cloth. The inoculum was then diluted 4 times with distilled water before it was used.

Techniques

Carborundum Method The first method used was the carborundum method which consisted of rubbing an area of a leaf gently with a glass rod after some 400-600 mesh carborundum and a drop of inoculum had been placed on it. (Plate 1. and 2.) The plants were shaded for twenty four hours prior to inoculation to increase the susceptibility.

"Dip method" The second method used has been named the "dip method" by the author and consists of dipping the bare roots of the plants into the inoculum at the time of transplanting. This method was not reported in any of the literature reviewed as a means of inoculating tomato plants with viruses. Though several workers reported that infection of certain plants could take place through the roots. It is similar to a method employed in inoculating plants with some

phytopathogenic fungi.

In preliminary tests, different time intervals were used and the results showed that dipping for a few minutes was equally as effective as allowing the plants to remain in the inoculum for two hour intervals. Longer periods of standing in the inoculum produced stem necrosis, that resembled damping off, described by Mogendorff (1930).

DISCUSSION OF RESULTS

In a problem dealing with resistance it is necessary to establish an arbitary set of standards. In this problem the following set of standards were used. When the percentage of plants that showed symptoms was: 0 percent, resistant; from 1 to 25 percent, slightly resistant; from 26 to 50 percent, slightly susceptible; from 51 to 75 percent, moderately susceptible, and 76 to 100 percent, highly susceptible. The percentage of infection has been shown in Table 2.

Commercial Varieties

None of the forty-two commerical varieties tested showed any resistance.

Species

The species tested varied widely in their reaction to the virus complex. Those species which offered no resistance were: the ten strains of L. esculentum; the four strains of the cross L. esulentum x L. pimpinellifolium, and the four

Table 2. Percentage of infection and number of plants used in trial.

Accession Number	Percentage of Infection (1)	No. of plants in trial (2)	No. of check plants
1	50.0	10	6
2	75.0	32	7
3	53.3	30	7
4	80.9	47	6
5	54.8	31	7
6	92.0	50	6
7	93.6	47	6
8	60.0	20	3
9	92.3	52	4
10	100.0	7	0
11	64.3	28	7
12	75.0	28	6
13	85.7	14	6
14	69.6	23	6
15	16.7	18	7
16	39.1	23	7
17	22.2	28	7
18	0.0	25	6
19	0.0	24	6
20	0.0	42	4
21	18.2	22	6
22	0.0	28	7
23	51.8	27	5
24	15.8	38	6
25	16.7	30	7
26	100.0	19	6
27	0.0	17	6
28	71.4	21	7
29	0.0	44	5
30	64.7	17	6
31	0.0	3	5
32	55.3	38	6
33	0.0	35	6
34	31.6	38	3
35	43.8	16	6
36	88.1	42	6
37	100.0	49	6

1. To the nearest tenth of a percent.

2. Includes only those inoculated plants living at the time the symptoms were read.

Table 2. (concl.) Percentage of infection and number of plants used in trial.

Acession Number	Percentage of infection (1)	No. of plants in trial (2)	No. of check plants
38	100.0	29	6
39	90.5	42	6
40	100.0	34	4
41	100.0	50	5
42	100.0	51	6
43	100.0	50	6
44	100.0	46	6
45	100.0	54	6
46	98.0	51	6
47	98.1	52	6
48	86.0	43	4
49	97.8	45	6
50	86.4	59	5
51	100.0	56	6
52	92.2	51	5
53	95.9	49	6
54	100.0	58	7
55	94.2	52	6
56	95.7	47	7
57	95.8	48	6
58	86.0	57	5
59	92.7	55	6
60	94.3	53	6
61	100.0	52	5
62	93.6	53	6
63	92.4	53	6
64	48.1	52	5
65	98.0	51	6
66	83.3	12	1
67	94.0	50	6
68	95.8	48	5
69	75.5	49	4
70	77.8	18	5
71	94.3	53	5
72	70.2	47	6
73	80.0	50	6
74	82.9	47	5
75	89.8	49	6
76	83.0	47	6
77	76.1	46	5
78	92.0	50	5
79	86.0	43	4
80	69.4	49	6
81	70.6	51	5
82	76.1	46	5

"marker-gene accessions." The following strains of the different species offered some resistance: L. pimpinellifolium PI 49532; PI 126939; PI 128639; and PI 143522; L. glandulosum PI 126434; L. peruvianum PI 126434; PI 126935; PI 128648; PI 129135, and PI 129152 and all four strains of L. hirsutum.

Methods of Inoculation

The "dip method" of inoculation used in this problem was found to be many times faster than the carborundum method. The percentage of infection in some instances was 100 percent, this would seem to substantiate its use in this problem.

During part of the experiment a mite infestation occurred. Inspection of plants after this infestation showed that symptoms appeared on all plants including the check plants. It would appear that the two spotted mite was capable of transmitting the disease. More work on this virus transmission is required before any positive statements can be made.

SUMMARY

1. Eighty-two species and commercial varieties of tomatoes were inoculated with a virus complex to determine if any resistance could be found.

2. None of the commercial varieties showed any resistance to the virus complex.

3. The species Lycopersicon esculentum showed no resistance to the virus comples.

4. The "marker-gene accessions" showed no resistance to the virus complex.

5. The species cross L. esculentum x L. pimpinellifolium showed no resistance to the virus complex.

6. The species L. hirsutum showed resistance to the virus complex.

7. The following species, L. pimpinellifolium, L. peruvianum, and L. glandulosum, showed marked differences in their reaction to the virus complex ranging from susceptible to resistance.

EXPLANATION OF PLATE I

Equipment used in the carborundum method of inoculation.

PLATE I

EXPLANATION OF PLATE II

Inoculating a leaf of a tomato plant by the carborundum method.

PLATE II

ACKNOWLEDGEMENT

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Abstract of a Thesis

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PURPOSE

The objectives of this experiment were---

1. To screen numerous species and horticultural varieties of tomatoes for resistance to a virus complex.

2. To incorporate this information into the tomato research program at Kansas State College.

METHODS

The plants tested were grown and inoculated by two different methods.

Methods of production were: (1) grown in flats in a raised bench. The spacing being two inches x three inches between plants with two varieties and/or species in each flat, (2) grown in a ground bench. The spacing being six inches between rows and one inch between plants in the row.

Methods of inoculation were: (1) the carborundum method which consisted of applying carborundum and a drop of inoculum to an area of a leaf and rubbing this area gently with a glass rod. (2) the "dip method" was also used and consists of merely dipping the roots of the seedlings into the inoculum at the time of transplanting.

The latter method has not been reported previously as a method of inoculating tomatoes with virus diseases but has been employed for the inoculation with some phytopathgenic fungi. While many workers have reported success in inoculating plant roots, few have ever succeeded in producing symptoms in the above ground portion of the plant.

RESULTS

1. No resistance was found in the commercial strains to the virus complex.

2. No resistance was found in the species Lycopersicon esculentum.

3. No resistance was found in the marker-gene accessions.

4. No resistance was found in the species cross L. pimpinellifolium x L. esculentum.

5. The species L. hirsutum showed resistance to the virus complex.

6. The following species, L. pimpinellifolium, L. peruvianum, and L. glandulosum showed marked differences in their reaction to the virus complex ranging from susceptible to resistant.

7. During an infestation of two spotted mite all plants having mites showed symptoms at a later date indicating that this insect could be listed as a suspected vector.